

**Effects of an 8-day advanced meditation,  
Samyama  
On physical, psychological and spiritual wellbeing,  
And associated neural mechanisms  
IRB #1801728792**

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## 1.0 Background

Neurotransmitters are the chemical (compounds) messengers produced by the body that regulate various activities in the brain and body. The human brain has its own neurotransmitter systems that play a crucial role in our cognitive and behavioral functions. Thus far, hundreds of neurotransmitters have been identified and more remain undiscovered. Among these, *Anandamide (AEA)* and *Brain-derived neurotrophic factor (BDNF)* are two neurotransmitters that are important in regulating mental mood and psychological wellbeing as well as physical health.

Samyama is an intensive eight-day residential program with meditations that provide the experiential possibility to free oneself from and purify the body-mind to receive higher levels of energy. Samyama presents the potential for participants to reach heightened levels of consciousness and experience explosive states of meditateness in the presence of a self-realized yogi. Samyama is a state where you reach heightened level of awareness. Many previous participants have reported feelings of bliss, intense joy, and oneness with others.

In this program, participants would have gone through other forms of meditation training from the same school of yoga. They are required to prepare themselves for over sixty days prior to coming to the 8-day silence treat. Preparatory steps include dietary regulations such as (vegan diet, adding more greens to their food in a stepwise manner), postural (Hatha) yoga practices, and meditation practices. At the silence treat, participants practice one point concentration with guidance. In the practice of concentration, which precedes meditation, participants learn how to slow down the thinking process by concentrating on a single object. The participants also learn to focus and watch their breath in closed eye states. Extended periods of concentration naturally lead to meditation. The Samyama program is offered once a year and has taken place for over 25 years and is executed under careful guidance of an advanced yogi.

## 2.0 Rationale and Specific Aims

Samyama participants often report a state of blissfulness, ecstasy, intense happiness, inclusive perception or higher states of consciousness during and after the program. The positive effects of contemplative practices such as yoga and meditation on mind-body wellbeing are well known and reported [1, 2]. However, the exact neural mechanisms through which each of these practices create these physiological and psychological beneficial effects are not completely understood.

The purpose of this study is to investigate the effects of Samyama program on blood levels of selected neurotransmitters before and after the program, and associate with corresponding effects on mind/psyche before and after the program in adult participants.

### Hypothesis:

1. The state of higher consciousness and ecstasy resulting from Samyama result from increased levels of Anandamide, an endocannabinoid, and Brain Derived Neurotrophic Factor (BDNF).
2. The 60-day preparatory phase that includes dietary regulation and yogic practices will reduce the gut inflammation and thereby will improve the gut microbiome.
3. **Assessment of the neurophysiological (EEG and MRI) changes with meditative breath watching before and after Samyama**
  - a) Changes in EEG patterns (frequency shift and source identification)
  - b) Changes in neuroconnectivity (default mode network activity)

### Part A:

**Anandamide (AEA)** activates endocannabinoid-1 (eCB1) receptors in the central nervous system and eCB2 receptors throughout the body and studies have suggested its role in regulating mood and behavior, sleeping and eating patterns, memory, pain, immune system, fertility and cancer inhibition [3]. Endogenous anandamide is mainly active at eCB1 receptor, which is responsible for the experience of blissfulness, ecstasy and the elevated mood and wellbeing similar to psychoactive effects reported in cannabis use. Anandamide is thought to be related to the experience of “runner’s high” due to intense physical activity [4]. So far, no study has explored the effect of yoga and meditation on endogenous levels of anandamide in humans. However, *Anandamide* is very quickly catabolized in the human system by Fatty Acid Amide Hydrolase (FAAH) [5]. So, to control for this we propose to use FAAH inhibitor in the sampling tube to preserve and accurately measure *anandamide*.

**Brain-derived neurotrophic factor (BDNF)** is another neurotransmitter found in the brain and the rest of the body. It helps in survival of existing neurons and growth of new neurons. It is involved in regulation of other neurotransmitters, long-term memory, stable memory function and cognitive function [6, 7]. Studies have suggested negative correlations between BDNF levels and disease such as depression, schizophrenia, obsessive-compulsive disorder, Alzheimer's disease, anorexia and dementia. Studies have shown beneficial increase in BDNF levels in human subjects because of yoga and meditation practices [8].

It is also expected that the participants will have varying responses of happiness and oneness within and between themselves to this advanced meditation program, Samyama. We hypothesize that the elevated mood and sense of wellbeing reported by Samyama participants could be correlated with one or more of the following factors:

1. Increased levels of Anandamide and/or BDNF levels in the bloodstream.
2. Decreased/delayed inhibition of these factors and hence, enhanced/prolonged effect (e.g., low levels of endogenous FAAH is associated with higher levels of anandamide).

3. Genetic variations (CB1, FAAH and other genes), and altered gene expressions, which would influence anandamide and FAAH levels and effects and function of CB1 receptors.

We will also explore other bio-markers and gene expressions (transcriptomics, DNA methylation, etc.) that are associated with bliss and oneness feelings of meditation.

**Microbiome analysis:** Participants will also be requested for stool samples prior to, or early into the start of preparatory steps (approximately 60 days before the scheduled program start), within 2.5 weeks prior to the program, and another one after the completion of the program. The strict vegan diet and the preparatory steps along with the Samyama meditation program is hypothesized to reduce the gut inflammation and improve the gut microbiome.

**Gut Inflammation analysis:** We will explore the relations between perceived stress, blood cortisol, plasma tryptophan catabolites (i.e., kynureine and quinolinic acid), and cytokines potentially related to UC (GM-CSF, Eotaxin, IFN- $\alpha$ , IP-10, IFN- $\gamma$ , MCP-1, IL-1 $\beta$ , MIG, IL-1RA, MIP-1 $\alpha$ , IL-2, MIP-1 $\beta$ , IL-2R, RANTES, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, and TNF- $\alpha$ )

**Transcriptome analysis (RNA sequencing – gene expression analyses):** Peripheral blood from participants will be collected before and after Samyama in PAXgene Blood RNA Tubes (Qiagen). Upon RNA extraction, RNA quantity will be assessed with Nanodrop (Nanodrop Technologies) and quality with the Agilent Bioanalyzer (Agilent Technologies). RNA samples will be cleaned using DNase I kit according to the Rapid out removal DNA kit instruction (Thermoscientific) and will be converted into cDNA by using Lexogen QuantSeq 3' Fwd Library Prep Kit (Lexogen) according to manufacturer's instruction to generate compatible library for Illumina sequencing. cDNA libraries will be assessed using TapeStation (Agilent Technologies, USA) before 65-100 bp single end sequencing using Illumina HiSeq 4000 system based on standard protocols to obtain raw sequencing data. Raw sequencing reads will be processed using our standard pipelines to obtain high quality sequencing reads for data analyses.

**Epigenetic analysis (DNA methylation):** DNA from peripheral blood of participants will be examined for integrity by agarose gel electrophoresis and quantified using fluorimeter using a double stranded DNA (BR) assay (Thermo Fisher Scientific). About 500 ng of the sampled DNA will be analyzed on a Infinium® MethylationEPIC BeadChip (Illumina, San Diego, CA). The hybridized and stained arrays will be ultimately scanned using HiScanSQ system (Illumina). Infinium MethylationEPIC (EPIC) BeadChip from Illumina will evaluate over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution. Data analyses will be performed utilizing previously published standard pipelines.

**Plasma lipidomic analyses:** A high-resolution (0.2 to 3 ppm mass error) mass spectrometric, non-targeted lipidomics platform will be utilized to compare the plasma

lipidome of Samyama participants before and after the program. Blood samples will be collected at the following three time points: (1) before the start of the Samyama program, (2) after the Samyama program, and (3) possibly at 3 months after the program. At each time point, one cc of blood will be drawn in purple top tubes and spun at 400 x g for 15 min at 4°C. The supernatant will be transferred to labeled cryovial and placed on dry ice immediately. Samples will be sent to the Lincoln Memorial University Lipidomics/Metabolomics Laboratory (422, Hamilton Math and Science Building, 6965 Cumberland Gap Parkway, Harrogate, TN) for analyses.

Lipids will be extracted with methy-tert-butyl ether and methanol containing [<sup>2</sup>H<sub>8</sub>]arachidonic acid, [<sup>2</sup>H<sub>4</sub>]hexacosanoic acid, [<sup>13</sup>C<sub>18</sub>]stearic acid, [<sup>2</sup>H<sub>28</sub>]DC 16:0, [<sup>2</sup>H<sub>7</sub>]cholesterol sulfate, [<sup>2</sup>H<sub>5</sub>]MAG 18:1, [<sup>13</sup>C<sub>3</sub>]DAG 36:2, [<sup>2</sup>H<sub>5</sub>]TAG 48:0, [<sup>2</sup>H<sub>31</sub>]PtdE 34:1, [<sup>2</sup>H<sub>54</sub>]PtdE 28:0, [<sup>2</sup>H<sub>31</sub>]PtdC 34:1, [<sup>2</sup>H<sub>54</sub>]PtdC 28:0, [<sup>2</sup>H<sub>62</sub>]PtdC 32:0, [<sup>2</sup>H<sub>31</sub>]SM 16:0, [<sup>2</sup>H<sub>31</sub>]PS 36:1, [<sup>2</sup>H<sub>31</sub>]PA 34:1, [<sup>2</sup>H<sub>62</sub>]PG 32:0, [<sup>2</sup>H<sub>3</sub>]carnitine 18:0, [<sup>2</sup>H<sub>4</sub>]LPC, CL(56:0), glyburide, and bromocriptine as internal standards. Extracts will be dried by centrifugal vacuum evaporation and dissolved in isopropanol : methanol : chloroform (4:2:1) containing 15 mM ammonium acetate. Non-targeted lipidomics (5 µL per min) will be performed utilizing high-resolution (140,000 at 200 amu) data acquisition, with sub-millimass accuracy on an orbitrap mass spectrometer (Thermo Q Exactive) with successive switching between polarity modes. Washes (500 µL) with methanol followed by hexane/ethyl acetate (3:2), between samples, are used to minimize ghost effects.

In negative ion ESI, the anions of ethanolamine plasmalogens (PlsE), phosphatidylethanolamines (PtdE), lysophosphatidylethanolamines (LPE), lysoalkenyl-acyl glycerophosphoethanolamines (LPEp), phosphatidylglycerols (PG), lysophosphatidylglycerols (LPG), phosphatidic acids (PA), lysophosphatidic acids (LPA), phosphatidylinositols (PI), lysophosphatidylinositols (LPI), phosphatidylserines (PS), lysophosphatidylserines (LPS), ceramides, N-acylphosphatidylserines (NAPS), N-acyl serines (NAS), fatty acids, dicarboxylic acids, very-long-chain fatty acids, and sulfatides, and the [M-2H]<sup>2-</sup> anions of cardiolipins (CL) will be quantitated and lipid identities validated by MS/MS with high resolution analyses of product ions.

In positive ion ESI, the cations of choline plasmalogens (PlsC), phosphatidylcholines (PtdC), lysophosphatidylcholines (LPC), sphingomyelins (SM), sphingosine, monoacylglycerols (MG), platelet-activating factors (PAF), acylcarnitines (ACar), ceramides, and the ammonium adducts of diacylglycerols (DG), triacylglycerols (TG), and cholesterol esters (CE) will be quantitated and lipid identities validated by MS/MS with high resolution analyses of product ions. The cations and anions of bromocriptine and glyburide are used to monitor for potential mass axis drift.

### 3.0 Inclusion/Exclusion Criteria

#### Inclusion:

1. Advanced meditation program participants age 18 or older (and interested spouses/significant others who live with meditation program participants.)

#### Exclusion:

1. Inability to read and comprehend the consent form
2. Subjects with medical conditions in which a blood draw would be contra-indicated (e.g. severe anemia).
3. Active marijuana/opioid and related drug use
4. Probiotic or prebiotic supplements within 60 days of enrollment
5. Antibiotic use within 60 days of enrollment
6. Participants living outside of the country.
7. Spouses who indicate in the online survey that they participate in meditation will be excluded from stool and blood samples.

#### **4.0 Enrollment**

Up to 200 participants, attending the Samyama Program will have the option to take part in a blood draw study to evaluate the serum levels of the biomarkers discussed.

Participants will be emailed an invitation letter with study information 2 months prior to the program, including a link to online surveys. Participants who respond that they would like to participate in either the blood or the stool sample will be asked for their home address and appropriate collection devices (for blood, stool or both) and instructions will be mailed to them for whichever options they decide.

Approximately 15 ml of blood will be collected four times per participant for this study. The first will occur at home (or at an Isha Center group meditation) prior to the program, the second sample at Isha Institute of Inner Sciences (IIIS) before the meditation program. Blood will also be collected (15 ml per draw) after the meditation program at Isha and again at home after program completion. All blood draws will be collected using standard sterile venipuncture methods by well-trained physicians, nurses and phlebotomists (please see enclosed list).

#### **5.0 Study Procedures**

##### Surveys Only Participants-

Participants will be invited to complete online surveys at following 4 time-points: Baseline (Time point 1), immediately pre-samyama (Time point 2), immediately after Samyama (Time point 3) and 3 months after Samyama (Time point 4). (Please see table below). Participants who are completing the surveys only (no blood draws) will be provided a Study Information Sheet describing the study at the beginning of the survey. We are requesting a waiver of consent signature for the survey only population. By answering the survey question, participants will be providing their consent to participate.

##### Surveys + Blood Draws Participants

Participants who choose to participate in the optional blood draws will have two blood samples drawn prior to beginning the advance meditation program and two blood draws

after completing the program (potential total of 4 blood draws). Approximately 15 ml of blood will be collected each time and separated into different sampling tubes for measuring blood levels of HbA1c, lipid profile, C-Reaction Protein, Cortisol, pro-inflammatory cytokines, AEA, endocannabinoid metabolites and BDNF (2 ml); genotyping and gene expression studies (3 ml) and the remaining 5 ml for future biomarker measurements. This blood sample will be labelled with a code for confidentiality of participants. The biomarker samples (AEA, BDNF) will be sent to Indiana University. Samples may also be sent off to other specialty labs for additional analysis including, but not limited to labs in Denver, Colorado; [Lincoln Memorial University Lipidomics/Metabolomics Laboratory \(422, Hamilton Math and Science Building, 6965 Cumberland Gap Parkway, Harrogate, TN\)](#); Gainesville, Florida; or San Diego, California. Genetic and future biomarker samples will be stored at the PI's Indiana University lab facilities.

Pre-preparatory period or early into the pre-preparatory period, (approximately 60 days before the program), immediately pre-Samyama (day of to up to 2.5 weeks prior) and 1-3 weeks post and 3-4 months post Samyama, different validated happiness, anxiety and depression scales (please see appendix) will be administered using electronic survey tools. Please see below for the links of surveys. CES-D, MAAS, PWB, EDA-SF8a, and Happiness scale will be administered in Pre-preparatory, Pre-Samyama and Post-Samyama (1-3 weeks post and 3-4 months post). Additional questions regarding the participants' gender, race, diet, yoga practice, bowel movements, allergies, medical conditions, medications, dietary restrictions and overall health/well-being will be included in the surveys. Those who agree to participate in the stool and/or at home blood draws will be asked for their contact information for scheduling blood draws and sending supplies.

#### Optional Stool Samples

For Microbiome analysis, stool samples will be collected via snail-mailed sample containers with stamped envelopes to be returned to the main study center and/or participants may bring the pre-program samples with them to Isha and give to study team member, or provide these samples to the phlebotomist drawing blood (if applicable). An instruction sheet will be attached to these boxes. Participation is entirely voluntary and it is not required that all participants in other arms of the study should also provide stool samples.

Fecal Microbiome Whole Metagenome Sequencing: Whole metagenomic sequencing (WMS) will be performed using the MiSeq Illumina platform as previously described<sup>50, 51</sup>. WMS allows for the identification of archaeal, fungal, bacterial, viral, and human DNA and predicts metabolic functionality. Although we acknowledge viruses and fungi also play a role in microbial ecology and response to diet, we will initially focus on microbial species and functional changes related to metabolite production (i.e., SCFA such as butyrate). Sequencing reads will be assembled into contigs with the Ray *de novo* assembler<sup>52</sup>. Reads from individual samples will be mapped to >5 kb assembled contigs using Bowtie-2<sup>53</sup>. Processing and visualization of mapped reads will be done using Anvi'o<sup>54</sup> which is a multifunctional platform for in-depth processing and exploration of 'omics datasets, with features including metagenomic binning, taxonomic classification, functional annotation, gene and metagenomic bin quantification, and data visualization. Anvi'o uses well-

established bioinformatics tools in the pipeline such as RAST<sup>55</sup>, HMMer<sup>56</sup>, and Prodigal<sup>57</sup>. SCFAs: SCFA will be measured in stool samples by gas chromatography. Serum Metabolomics: Metabolomics analysis provides a snapshot of an organism's current metabolite profile. Sample preparation and metabolomics analysis will be performed according to our previous work<sup>58</sup>. Briefly, serum samples will be prepared, including filtering and pH standardization and then all nuclear magnetic resonance (NMR) experiments performed on a Bruker Advance 600 spectrometer (Bruker Biospin, Milton, Canada). Processed spectra will be imported into Chenomx NMR Suite software (Edmonton, AB) for metabolite identification and quantification. To describe the metabolite changes in the context of other physiological changes, metabolomics data will be integrated with the other biological variables assessed using O2PLS-DA (orthogonal partial least squares discriminatory analysis) modeling<sup>58,59</sup>.

**Serum Inflammatory Cytokines:** Serum cytokines will be measured using a multiplex commercial flow-based 25 cytokine assay (Luminex, Thermo Fisher). Cytokines to be measured are GM-CSF, Eotaxin, IFN- $\alpha$ , IP-10, IFN- $\gamma$ , MCP-1, IL-1 $\beta$ , MIG, IL-1RA, MIP-1 $\alpha$ , IL-2, MIP-1 $\beta$ , IL-2R, RANTES, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, and TNF- $\alpha$ .

#### **Baseline/Pre-preparatory Samyama (all to occur prior to Feb. 22<sup>nd</sup>)**

- Online Surveys to be completed before Feb 22<sup>nd</sup>
- Survey link for participant: <https://www.surveymonkey.com/r/6TK3V5K>
- Survey link for spouse/ "control": <https://www.surveymonkey.com/r/6NX5N3L>
- Stool collection from home
- Blood sample at home provided by at home phlebotomy services or at Isha Center group meditation by study personnel.

#### **Post-Preparatory/Pre-Samyama (to occur Mar 15<sup>th</sup> to Mar 31<sup>st</sup>)**

- Online Surveys
  - Participant: <https://www.surveymonkey.com/r/XWBS8PM>
  - Spouse: <https://www.surveymonkey.com/r/XTM6K5S>
- Stool collection from IIS/home
- Blood sample collection: on site at IIS/home from **March 24<sup>th</sup> to March 31<sup>st</sup>**

#### **Immediate Post-Samyama (April 7<sup>th</sup> to April 21<sup>st</sup>)**

- Online survey to be completed between April 23<sup>rd</sup> and May 23<sup>rd</sup>
- Survey link for participant: <https://www.surveymonkey.com/r/6TGSTNL>
- Survey link for spouse: <https://www.surveymonkey.com/r/6FDWCDT>
- Blood sample collection: on site **at IIS** on **April 7<sup>th</sup> evening and April 15<sup>th</sup>**
  - Participant only – Not spouse/significant other.

### Post Samyama (Jul 7<sup>th</sup> and Jul 31<sup>st</sup>)

- Blood draw at home by at home phlebotomist
- Post program online survey follow up to be completed
- Survey link for participant: <https://www.surveymonkey.com/r/6MKH277>
- Survey link for spouse: <https://www.surveymonkey.com/r/6FQJ523>
- Stool collection from home

<b>Time points</b>	<b>Online Surveys</b> Time requirement: 20 minutes each	<b>Blood sample Collections</b> Time required: 10 minutes each	<b>Stool sample collections</b> Time required: 10 minutes each
<b>First: Baseline (Before Feb 22<sup>nd</sup>)</b>	Survey #1: <a href="https://www.surveymonkey.com/r/6TK3V5K">https://www.surveymonkey.com/r/6TK3V5K</a>	At Home or City	At Home/ Isha Center
<b>Second: Immediate pre-Samyama (March 15<sup>th</sup> to March 31<sup>st</sup>)</b>	Survey #2: <a href="https://www.surveymonkey.com/r/XWBS8PM">https://www.surveymonkey.com/r/XWBS8PM</a>	At Isha Institute of Inner Sciences	At Home/III
<b>Third: Immediate Post- Samyama (April 7<sup>th</sup> to April 21<sup>st</sup>)</b>	Survey #3: <a href="https://www.surveymonkey.com/r/6TGSTNL">https://www.surveymonkey.com/r/6TGSTNL</a>	At Isha Institute of Inner Sciences	-
<b>Fourth: 3 months after Samyama (July 7<sup>th</sup> and July 31<sup>st</sup>)</b>	Survey #4: <a href="https://www.surveymonkey.com/r/6MKH277">https://www.surveymonkey.com/r/6MKH277</a>	At Home or City	At Home

### 6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Venipuncture will be performed by trained personnel in order to minimize risk. The principal investigator will be present at the site, and notified of and closely monitor any adverse events occurring during the study. All unexpected, related or possibly related to participation in the research serious adverse events will be reported to the IRB promptly after the site becomes aware of the event. Emergent medical events associated with blood draws if any (e.g. vasovagal episodes) will be timely managed by the PI and other physician volunteers at IIS and reported immediately to the IRB. The PI will be available for medical help as well as an on-site health clinic in the event an adverse event occurs.

## **7.0 Study Withdrawal/Discontinuation**

A participant may withdraw at any time from the study by notifying the research staff. Participation in this study is voluntary. Participation in the blood draw part of the study is optional as is the stool sample. Participants who did not participate in the at home pre-blood draw may still choose to take part in the 2 blood draws (before and after Samyama Program) at IIS. Only those participants who had pre- at home blood draws will be invited to have the post program at home blood draws.

## **8.0 Statistical Considerations**

This is a preliminary study on the endocannabinoid system's changes with meditation. Such a study has not been done previously. We will do descriptive statistics comparing before and after Samyama biomarkers, genetic differences and correlate changes with the participants before and after Samyama questionnaire data. This study's data will be used to develop additional hypothesis for future larger studies to objectively show physiological and psychological benefits of meditation.

## **9.0 Privacy/Confidentiality Issues**

Blood samples will not have PHI associated with them. Unique codes will be assigned to de-identify participants and materials that are shared with sources outside of the research team involved in the study.

## **10.0 Follow-up and Record Retention**

Two Post-Samyama electronic questionnaire follow-ups will be done via email as well.

Records will be maintained by the PI and research team. De-identified data will be used for data analyses, and publications.

## **Part B:**

The primary aim of Part B of the study is to study the changes in EEG patterns during breath watching associated with the 8-days Samyama program. fMRI will be used to qualitatively describe the anatomical changes and functional changes including the DMN activity associated with breath watching before and after the Samyama program.

**EEG:** Studies have shown that EEG can be used to explore the temporal relation between measures of functional connectivity network integration and meditation quality<sup>15</sup>. van Lutterveld et al studied the functional connectivity with the help of Minimum spanning tree (MST) and graph measures. It has high temporal resolution of the order of milliseconds.

Studies have also shown that meditation produces different activities at different locations such as increased alpha activity in frontal regions<sup>16</sup> and posterior regions<sup>17</sup>. A meta-analysis conducted in 2014 showed the most consistently altered brain regions were the left rostrolateral prefrontal cortex, anterior/mid cingulate cortex, anterior insula, primary/secondary somatomotor cortices inferior temporal gyrus, and hippocampus<sup>18</sup>. Insula is consistently linked to interoception-awareness of the body's internal and visceral states, including respiration, heart rate, emotional self-awareness, and potentially metacognitive awareness<sup>19,20</sup>. Somatosensory areas are also altered by meditation.

Examine the EEG changes associated with 8-day advanced meditation, Samyama program. This includes,

- EEG patterns at rest
- EEG patterns during Meditative Breath watching
- EEG patterns during 'Shoonya' meditation

**fMRI:** Functional MRI is a useful non-invasive tool to study brain neuronal activity. Several resting state functional MRI (rs-fMRI) networks have been described. These networks are temporally connected regions of brain activation. One of the major rs-fMRI networks is the Default Mode Network (DMN), which has shown changes with meditation training<sup>21</sup>.

Default-mode network (DMN) refers to the neural activity in a network of brain areas that support self-referential processing<sup>22</sup>. The group of brain regions that has collectively been termed the "default network" includes, most prominently, the medial prefrontal cortex (PFC), posterior cingulate cortex/precuneus region, and the temporoparietal junction<sup>23</sup>. This network has been associated with processes ranging from attentional lapses to anxiety to clinical disorders, such as attention-deficit hyperactivity disorder (ADHD) and Alzheimer's Disease<sup>24, 25</sup>. Gilbert et al. recently argued that activations in the medial PFC part of the default network may reflect stimulus-related thought such as enhanced watchfulness toward the external environment that is also likely to occur during highly practiced tasks<sup>26</sup>. We postulate that there will be decrease in the default mode network activity after the Samyama program with increase being in the moment experiences.

Examine the qualitative fMRI changes associated with 8-day advanced meditation, Samyama program. This would specifically examine the resting state neuroconnectivity, in particular the 'default mode network'. Anatomical changes such as grey matter volume and insular thickness will also be analyzed. fMRI changes with meditative breath watching will also be recorded and analysed.

## **11.0 Inclusion/Exclusion Criteria**

### Inclusion:

- 1) Advanced meditation program participants who are healthy adults of age 18 or older
- 2) Healthy adult non-meditators over 18 years for controls

### Exclusion:

- 1) History of any neurological condition (i.e. Parkinson's disease, Alzheimer's disease, Huntington's disease, brain tumors, brain surgery, or multiple sclerosis)
- 2) History of depression, currently being treated with antidepressants
- 3) History of any psychiatric disorder, within last 5 years (i.e. anxiety, psychosis, posttraumatic stress disorder, attention deficit hyperactive disorder)
- 4) Current use of cognition enhancing medications
- 5) Active history (within the last 5 years) of alcohol or drug abuse (> 10 drinks per week)
- 6) History (within the last 5 years) of stroke/aneurysm
- 7) Recent history (< 3 months) of seizures
- 8) History of claustrophobia
- 9) History of metal or electronic implants
- 10) Pregnancy

## **12.0 Enrollment**

Recruitment will occur from the interested subjects of Indianapolis and nearby states who will be participating in the Samyama program in Isha Institute of Inner-sciences, Tennessee, USA<sup>27</sup>. Recruitment will occur via communication modalities (email communications to participants and controls through the Isha Foundation that conducts this Samyama program) used by this particular school of yoga.

Subjects will be identified by the study team via email communications through the Isha Foundation.

When interested subjects contact the study team, the study will be discussed and the subject will undergo prescreening for eligibility via phone. If the subject meets eligibility criteria, an appointment for the first EEG and fMRI will be scheduled.

### **Study procedures:**

Functional brain imaging and EEG studies will be carried out between **March 12 and March 29, 2018 (before Samyama), and between April 9<sup>th</sup> and May 9<sup>th</sup>, 2018 (after Samyama)**

#### **1) EEG**

Subjects will undergo a resting EEG at baseline. This will occur within a time window of 2 weeks prior to the Samyama retreat. EEG will again be repeated within a month after the 8 day retreat.

The EEG recording will occur in 4 phases, with and without task engagements, which will occur in alternative sequence. The tasks in this particular study will be slow meditative

slow breathing and ‘Shoonya’ meditation. The following sequence will be instructed to the study participants during the EEG recording:

- 1) 10 minutes of rest
- 2) 10 minutes of breath watching as it happens during their Stage 4 Shambhavi meditation
- 3) 10 minutes of rest
- 4) 10 minutes of ‘Shoonya’ meditation (optional as it requires empty stomach conditions)

Meditative Slow Breathing is a common practice within yoga and some meditative practice traditions; it is known to reduce psychophysiological arousal and stress activation, specifically reducing sympathetic drive, enhancing parasympathetic activation, increasing heart rate variability, reducing emotional activity, and improving calmness and well-being. ‘Shoonya’ meditation is an effortless process of conscious undoing.

The ENOBIO EEG device is an FDA approved device under US federal law which is a 24-bit EEG data reader at the rate of 500 S/s which comes as a wearable wireless electrophysiology sensor system. It includes spectrogram and 3D visualization in real time for spectral features. It also collects triaxial accelerometer data. These data are stored in microSD offline in Holter mode<sup>28</sup>. This EEG communicates via wireless allowing the subjects to be in a comfortable position and also can reduce the effects of electrical interference.

## 2) Functional Brain Imaging (fMRI)

### fMRI Data Acquisition:

The anatomical and functional imaging will be performed using 3 Tesla Siemens PRISMA scanners using a 32 channel head coil. Imaging, including set up time will be approximately 30 min. Imaging sequences are as follows:

	Sequence	Parameters
Anatomic sequence	T1-weighted sagittal	MPRAGE, TR/TE=2010/2.91ms, flip angle=9°, field of view=192x174 mm, 192 sagittal slices, slice thickness=1mm, isotropic voxel size of 1 mm, Time=2:38min
RS-fMRI	Rs-fMRI – T2*-weighted EPI	Axial, TR = 1205/30ms, TE = 29 ms, flip angle= 50°, 51 slices, slice thickness= 2.5 mm, FOV= 180x180 mm <sup>2</sup> , isotropic voxel 2.5 mm, 500 volumes, Time=10min

MRI images from the scanner will be deidentified and stored in the Center for Neuroimaging repository.

### rs-fMRI analysis:

Standard preprocessing pipeline will be applied to remove non-brain tissue signal, realign and spatially normalize to the UNC brain template space,<sup>29</sup> and smoothed using FSL<sup>30</sup> or SPM12 (Cambridge, UK). We will use seed based regions of interest to identify the DMN.

	<b>Baseline</b>	<b>After 8 day Samyama program</b>
EEG	*	*
fMRI	*	*

### **RISKS OF TAKING PART IN THE STUDY**

There is a potential risk of loss of confidentiality. We will take precautions to keep the information secure.

The MRI scan is a safe procedure with minimal risks. The MRI scanner uses a powerful magnet to obtain pictures of the body instead of X-rays. Subjects will be asked questions before the MRI to ensure that there is no reason to prevent them from having the scan.

Due to the fields, which are present in the MRI scanner, loose magnetic objects (pocket knives key chains, necklaces, earrings, etc.) can fly into the magnet with great force if brought into the environment of the MRI scanner. Subjects will be asked to remove such objects from their clothes and body to prevent such an occurrence. There are some individuals who should not have an MRI scan. These include persons with some types of metallic implants, such as aneurysm clips or some types of prostheses (fake body parts), or persons with electronic implants, such as cardiac pace makers. The magnetic field in the scanner can cause displacement or malfunction of these devices.

Women who are pregnant should not participate. If subject is a woman of child-bearing potential, subject would be advised that the risks of MRI to a fetus are possible, but as yet unidentified.

Other potential risks are:

Collision hazard. The magnetic field near the MR scanner is strong enough to attract objects containing iron with great force. Near the magnet, this force can be strong enough to pull objects in and cause them to fly down into the magnet. Such objects can become projectiles that can cause injury or death. We have established a security zone to prevent objects containing iron from coming into proximity of the magnet.

Radio-wave effects. If metal wires or electrodes, such as electrocardiograph (ECG) leads, are attached to a person being imaged, the radio-wave energy radiated by the imaging coils of the MRI scan may induce sufficient electrical currents in the wires to

cause burns where the electrodes or wire contact the skin. The scanner operator is well aware of this risk and knows the proper methods to use to avoid this problem.

Nerve Stimulation. Some subjects undergoing the rapid scanning procedures, which will be used in this scan have experienced minor nerve stimulation effects, such as muscle twitches and tingling sensations. There are no known risks associated with these effects. The devices used in our research create varying magnetic fields that are within the limits specified by the Food and Drug Administration (FDA).

Claustrophobia. The confining conditions of the MR scanner can cause claustrophobia (the feeling of being "closed in") in some people. If subjects experience claustrophobia during the scan, they can request the scan be stopped.

Hearing: The MRI scanner produces tapping sounds during operation, which may reach objectionable levels. To minimize any discomfort, subjects will be provided with noise reducing disposable earplugs or headphones.

EEG: Noninvasive EEG monitoring is considered a safe procedure. It has no associated discomfort, pain or electric shock. In rare instances, EEG can precipitate seizures in individuals with a seizure disorder due to flashing of lights or deep breathing. Participants with history of seizures in last 3 months will not be recruited to minimize the risk. EEG will be performed by trained study staff using a wearable wireless electrophysiology sensor system.

### **13.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others**

The study interventions are non-invasive and minimal risk. Therefore, we will limit the scope of AE monitoring and reporting to the following:

- All Serious Adverse Events believed to be related to the study procedures
- Unexpected, non-serious Adverse Events believed to be related to the study procedures

### **14.0 Statistical Considerations**

- a. Sample Size Justification: Participants and volunteers from IN and nearby Indiana locations will be identified from information obtained from the Isha foundation. This observational exploratory analysis will include 15 volunteer meditators to study the EEG changes. 10 meditators who do similar preparatory meditations but not participate in Samyama will serve as the control subjects for fMRI studies. fMRI will be performed on 15 meditators and 10 controls who meditate similarly but not participate in Samyama.
- b. Data Analysis: Continuous data will be reported as means  $\pm$  standard deviation or median (quartile 1, quartile 3) depending on distribution. Normality will be assessed

with the Shapiro-Wilk test. Categorical data will be reported as frequencies and proportions and assessed with a chi-square or Fisher's exact test as appropriate. SAS 9.4 (SAS Institute Inc., Cary, NC) will be used for all analyses, with two-sided p-values < 0.05 considered statistically significant.

### **15.0 Study Withdrawal/Discontinuation**

A participant may withdraw at any time from the study by notifying the research staff. Participation in this study is voluntary.

### **16.0 Use of Scans**

MRI and EEG data from Indiana University and a collaborating site, part of Harvard University will be combined to increase the statistical power. De-identified MRI and EEG data will be mutually shared with Boston investigators (PI: Dr. Balachundhar Subramaniam).

Individual MRI results will not be made available to participants.

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32. *fMRI*.

## Appendix

- **Mindfulness** will be measured by the Mindful Attention Awareness Scale (MAAS) (5-Item) Mindful Attention Awareness Scale (MAAS) (Brown & Ryan, 2003; Osman, Lamis, Bagge, Freedenthal, & Barnes, 2016). Osman et. al. (2016) find that the 5-Item (below) is as reliable as the original MAAS. The Cronbach's alpha score of the 5-item is .88. The 5-item scale is as follows:

How often do you feel the following ways?

1. It seems I am "running on automatic," without much awareness of what I'm doing.
2. I rush through activities without being really attentive to them.
3. I get so focused on the goal I want to achieve that I lose touch with what I'm doing right now to get there.
4. I do jobs or tasks automatically, without being aware of what I'm doing.
5. I find myself doing things without paying attention.

(The response will be coded on a 6-point scale from 1 Almost Always to 6 Almost Never)

- **Joy** will be measured by the Joy Subscale of the Dispositional Positive Emotion Scales (6-Item) (Shioata, Keltner, & John, 2006). The Cronbach's alpha is .82.

1. I often feel burst of joy.
2. I am an intensely cheerful person.
3. I am often completely overjoyed when something good happens.
4. On a typical day, many events make me happy.
5. Good things happen to me all the time.
6. My life is always improving.

(The response will be coded on a 7-point Likert scale – 1 Strongly Disagree to 7 Strongly Agree)

- **Vitality** is "the state of feeling alive and alert--to having energy available to the Self" (Ryan & Frederick, 1997). Ryan and Frederick (2001) considers vitality as an aspect of eudaimonic well-being and suggests that "as being vital and energetic is part of what it means to be fully functioning and psychologically well." Vitality will be measured by the Subjective\_Vitality Scale (6-Item) (Bostic, Rubio, & Hood, 2000; Ryan & Frederick, 1997). Bostic et. al (2000) find that the 6-item is a better scale with Cronbach's alpha of .84.

Please indicate the degree to which the statement is true for you in general in your life.

1. I feel alive and vital.
2. Sometimes I feel so alive I just want to burst.
3. I have energy and spirit.
4. I look forward to each new day.
5. I nearly always feel alert and awake.
6. I feel energized.

(The response will be coded on a 7-point scale from 1 Not at all true to 7 Very True)

- **Forces of Spirituality** includes five sources: Transcendent, Theistic, Self, Nature, Human Oneness Within is measured by the Self subscale of the Sources of Spirituality Scale (Davis et al., 2015). The Cronbach's alpha ranges from .88 to .90.

1. I felt near to God
2. I felt close to God
3. I knew that God was with me
4. I sensed God's presence
5. I had a sense of something infinite
6. I felt a bond with an indescribable force of being
7. I felt a sense of oneness with something I cannot describe with words
8. I sensed something from another realm or dimension
9. I felt entirely authentic
10. I had a sense of integrity
11. I felt completely genuine
12. I had a sense of wholeness
13. I felt close to nature
14. I felt connected to nature
15. I felt near to nature
16. I felt connected to all of humanity
17. I felt near to all of humanity
18. I felt as if I were one with humanity

(The response will be coded as 1=Strongly Disagree to 5=Strongly Agree)

- **Flourishing Scale**

1. I lead a purposeful and meaningful life.
2. My social relationships are supportive and rewarding.
3. I am engaged and interested in my daily activities.
4. I actively contribute to the happiness and well-being of others.
5. I am competent and capable in the activities that are important to me.

6. I am a good person and live a good life.
7. I am optimistic about my future.
8. People respect me.

(The response will be coded as 1=Strongly Disagree to 5=Strongly Agree)

- **The 7-Item Short Nonattachment Scale**

To help us understand your general approach to life and your views about yourself, others, and life in general, tell us the extent to which the following statements reflect your experiences **at this point in your life**. Select a number from 1 to 6 on the scale provided with each statement to rate the extent to which you agree with it.

Please answer according to what **really reflects** your experience rather than what you think your experience should be.

1	2	3	4	5	6
Disagree Strongly	Disagree Moderately	Disagree Slightly	Agree Slightly	Agree Moderately	Agree Strongly

1. I can let go of regrets and feelings of dissatisfaction about the past.
2. I can enjoy pleasant experiences without needing them to last forever.
3. I view the problems that enter my life as things/issues to work on rather than reasons for becoming disheartened or demoralized.
4. I can enjoy my family and friends without feeling I need to hang on to them.
5. I can take joy in others' achievements without feeling envious.
6. I do not get "hung up" on wanting an "ideal" or "perfect" life.
7. When pleasant experiences end, I am fine moving on to what comes next.

- **Compassionate Short Scale**

1. When I hear about someone (a stranger) going through a difficult time, I feel a great deal of compassion for him or her.
2. I tend to feel compassion for people, even though I do not know them.
3. One of the activities that provide me with the most meaning to my life is helping others in the world when they need help.
4. I would rather engage in actions that help others, even though they are strangers, than engage in actions that would help me.
5. I often have tender feelings toward people (strangers) when they seem to be in need.

(From 1=Not at all true of me and to 7=Very true of me)

- **Center for Epidemiologic Studies Depression scale (CES-D-10)**

1. I was bothered by things that usually don't bother me.
2. I had trouble keeping my mind on what I was doing.
3. I felt depressed.
4. I felt like everything I did was an effort.

5. I felt hopeful about the future.
6. I felt fearful.
7. My sleep was restless.
8. I was happy.
9. I could not get going.

- **Brief Resilience Scale (BRS)**

1. I tend to bounce back quickly after hard times
2. I have a hard time making it through stressful events.
3. It does not take me long to recover from a stressful event.
4. It is hard for me to snap back when something bad happens.
5. I usually come through difficult times with little trouble.
6. I tend to take a long time to get over set-backs in my life.

Scoring:

Add the responses varying from 1-5 for all six items giving a range from 6-30. Divide the total sum by the total number of questions answered.

My score: \_\_\_\_\_ item average / 6

- Emotional Distress – Anxiety – Short Form 8A

PROMIS Item Bank v1.0 – Emotional Distress – Anxiety – Short Form 8a

**Emotional Distress – Anxiety – Short Form 8a**

Please respond to each question or statement by marking one box per row.

**In the past 7 days...**

		Never	Rarely	Sometimes	Often	Always
EDAN001	I felt fearful.....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN002	I found it hard to focus on anything other than my anxiety .....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN003	My worries overwhelmed me.....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN004	I felt uneasy .....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN005	I felt nervous.....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN006	I felt like I needed help for my anxiety .....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN007	I felt anxious.....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
EDAN008	I felt tense .....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5